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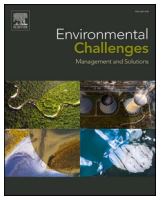

Current frameworks for the toxicological assessment of pesticide transformation products in drinking-water

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ABSTRACT

Despite the benefits of pesticides in agriculture, their widespread use contributes anthropogenic chemicals to the environment, sometimes resulting in the formation of pesticide transformation products. These transformation products may enter sources of drinking-water, potentially posing health risks when present at significant concentrations. Currently, there is no globally harmonized framework for the toxicological assessment of pesticide transformation products in drinking-water. The World Health Organization provides international guidance through the Guidelines for Drinking-water Quality. However, there remains a specific need for a more detailed framework addressing pesticide transformation products in drinking-water.

This review was conducted under contract with the World Health Organization to support the development of a consistent and transparent global assessment framework for pesticide transformation products in drinking-water. The aim was to identify and compare relevant assessment schemes, particularly those focused on transformation products in drinking-water or its sources, to evaluate their strengths and limitations, and to formulate recommendations for future international frameworks.

The analysis identified two primary guidance documents relevant to drinking-water: the European Commission's SANCO/221/2000 guidance, which varies in application across EU member states, and the ECHA/EFSA guidance focused on water treatment processes. Other frameworks, including those from the United States and the World Health Organization, lack provisions specific to transformation products in water. Common elements identified across frameworks include tiered assessment, comparison with parent pesticide toxicity, predictive modelling, aggregate exposure assessment, and consideration of treatment effects. The review highlights the need for an internationally harmonized approach to ensure consistent and effective management of transformation products in drinking-water.

1. Introduction

From a global perspective, pesticides are integral to modern agriculture, playing a pivotal role in enhancing food production by controlling pests that threaten crops. Their widespread use is essential for meeting the growing global demand for food. However, this

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extensive use also presents significant challenges. While pesticides protect crops, they introduce anthropogenic chemical substances into the environment, that may lead to unintended consequences. One major concern is the presence of pesticide transformation products in freshwater sources, including drinking-water sources, because water is a fundamental resource for life (Huang and Li, 2025; Tang et al., 2025).

In the context of a toxicological assessment of pesticide transformation products in drinking-water, it is necessary to consider all potential transformation and breakdown products, regardless of their origin. However, endogenous metabolites (those formed in the human body) fall outside the scope for this review. Within this review, the following terms are used, as defined here:

Transformation Products: A general term referring to all pesticide breakdown products, regardless of origin.

Metabolites: A subset of transformation products formed by biological processes in the environment, e.g., soil bacteria, within plants and/or animals.

Residues: A subset of transformation products formed by biotic and abiotic processes that are found in or on food and/or feed.

Water treatment transformation products: A subset of transformation products formed as a result of physico-chemical reactions of the active substance or precursor transformation products during water treatment processes, e.g., chlorination, ozonation, UV irradiation.

Degradates: A subset of transformation products formed by abiotic processes in the environment, e.g., photodegradation.

It is acknowledged that there is some over-lap in these definitions, e.g., formation via UV photolysis may occur during water treatment and in the environment. Alternate definitions and terms, as given in other, specific guidance documents are listed in Supplementary Table 1.

Chemical contaminants in drinking-water, depending on their inherent characteristics and exposure concentrations, can contribute to chronic health issues, including cancer, endocrine disruption, and reproductive and developmental problems. With regard to human health risk assessment, several frameworks and guidance documents have been developed to weigh these concerns. Within the EU for example, the European Union's SANCO/221/2000 guidance document is used for evaluating the toxicological relevance of pesticide transformation products in groundwater (European Commission, 2021) and the recently published ECHA/EFSA guidance document addresses the impact of water treatment processes on transformation products (EFSA and ECHA, 2023). The US Environmental Protection Agency (EPA) addresses "residues of concern" in drinking-water in part by utilizing both monitoring data and predictive models to assess potential risks (US EPA, 2013). At an international level, the WHO Guidelines for Drinking-water Quality (GDWQ) provide a reference point for the development of drinking-water quality regulations worldwide (WHO, 2022), with some specific guideline values derived from JMPR toxicological assessments.

Applying the precautionary principle, the EU Drinking-water Directive 2020/2184 and Groundwater Protection Directive 2006/118/EC specify general threshold concentrations of 0.1 μg/L for individual pesticides and 0.5 μg/L for the sum of pesticides and their relevant transformation products in drinking-water. The DWD further states that "*a pesticide metabolite* [i.e. transformation product] *shall be deemed relevant for water intended for human consumption if there is reason to consider that it has intrinsic properties comparable to those of the parent substance in terms of its pesticide target activity or that either itself or its transformation products generate a health risk for consumers.*" Non-relevant transformation products are subject to guidance values defined by individual Member States. Similarly, the US EPA uses its own threshold values and approaches for assessing pesticide transformation products in drinking-water.

Although the WHO has established an assessment scheme to evaluate pesticide dietary residues in plants, animal tissues and processed foods as part of the JMPR (WHO, 2015), the GDWQ lacks an assessment framework, not only for pesticides but also their transformation products, as there is no framework for actively seeking out transformation products. Internationally disparate assessments of pesticide transformation products may be the result, leading to global differences in acceptable limits in drinking-water and drinking-water sources. Indeed, even within the EU, which has a guidance document, the assessment of non-relevant transformation products is inconsistent, with different EU member states employing different limits (reviewed by Laabs et al., 2015).

Several water treatment strategies have long been known to have the potential to transform some pesticides into more toxic transformation products, the archetypal example being chlorination of water containing organophosphate pesticides, which results in the production of far more potent transformation products, namely oxons, sulfoxides, sulfones and/or oxon-sulfones (Fig. 1). Consideration of effects of water treatment on pesticides and their transformation products is limited (Li et al., 2016).

From a regulatory perspective, reference is made to these considerations in the US EPA Guidance on Incorporation of Water Treatment Effects on Pesticide Removal and Transformations in Drinking-water Exposure Assessments (US EPA, 2011) and, more recently, the EFSA/ECHA Guidance Document on the Impact of Water Treatment Processes on Residues of Active Substances or their Metabolites in Water Abstracted for the Production of Drinking-water (EFSA and ECHA, 2023).

The aim of the current review was a) to identify existing assessment schemes specifically for pesticide transformation products in drinking-water or sources of drinking-water and b) to identify existing toxicological assessment frameworks potentially relevant for the assessment of said transformation products, c) to review their strengths and limitations and d) to derive recommendations for a future global assessment framework that may ultimately be used in conjunction with the GDWQ for more consistent and transparent hazard and risk assessments.

2. Methodology

2.1. Search strategy and framework identification

A search using key word terms as well as direct contact with selected regulatory institutions and authorities was used to identify relevant existing frameworks. Google scholar, Web of Science and PubMed were used to search for published literature and information. The publications were reviewed manually based on the title and/or abstract. The following government websites providing information in the English language were also searched: US Environmental Protection Agency (EPA), Australian Pesticides and Veterinary Medicines Authority (APVMA), Health Canada, and China Ministry of Ecology and Environment (MEE). These government websites were selected to provide

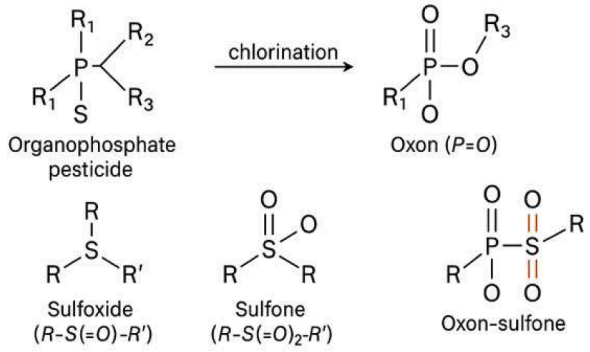

Fig. 1. General molecular structures of organophosphate pesticides and their transformation products formed by chlorination. (Generated using Perplexity AI).

coverage of non-European countries, as most European nations follow the SANCO guidance. This approach allows for a broader comparison of regulatory practices beyond the European context. Also, these agencies provide publicly available English-language guidance documents critical for comparative analysis. Although many other countries maintain strong and well-established regulatory frameworks, this review focused on a select number of jurisdictions to allow for a more detailed and manageable analysis.

The search was conducted using the following keywords in English only:

Pesticide transformation products
Pesticide metabolites
Pesticide residues
Pesticide degradation products
Pesticide degradates
Drinking-water
Groundwater

Combinations of these keywords were also used. This combination involved the chemical substance type (e.g., pesticide transformation products) and water type (e.g., drinking-water or groundwater). The various search combinations used are provided in Supplementary Table 2.

An advanced search was also performed due to the large number of initial results, using quotation marks (“ ”) and Boolean operator OR. In addition, manual refinement using filtering terms ("framework," "guidance," "hazard assessment," "risk assessment") was applied to obtain specific information. Also, relevant words or phrases such as hazard assessment, hazard identification, risk assessment were considered when examining the title and/or abstract of the publications or document.

2.2. Search for, and direct contact with, evaluating agencies and authorities

In order to expedite the process of searching for framework or guidance documents and due to the limited number of frameworks and guidance documents retrieved from various databases, various countries and their respective relevant authorities were contacted directly. Initially country authorities were identified based on a publication by Li and Fantke (2022), where countries that had freshwater quality standard for pesticides were listed. Additional countries were included in the list based on other significant factors related to pesticide concerns. For example, all the African countries added were based on the countries with the highest importation of pesticides (Heinrich Böll Stiftung, 2023). Countries that were contacted (Fig. 2) directly are given in Supplementary Table 3.

WHO regional office focal points, selected expert members of JMPR (Joint FAO/WHO Meeting on Pesticide Residues), and regulatory toxicologists from selected manufacturers of pesticide active substances and plant protection products were also contacted to obtain information on existing assessment frameworks and their implementation. A summary of the methodology is provided in Fig. 3.

3. Results and discussion

3.1. Regulatory frameworks identified

The initial search strategy yielded hundreds to thousands of results from both academic and governmental sources (Supplementary Table 2). This underscores the extensive literature available on pesticide transformation products and drinking-water. Nonetheless, manual review of the results resulted in the identification of two guidance documents namely SANCO/221/2000, which is specifically for the assessment of pesticide “metabolites”, i.e., transformation products in water, and the more recent EFSA/ECHA guidance document which addresses the impact of water treatment processes on residues mentioned above. Additional, potentially relevant assessment frameworks, were also identified (refer to Table 1), however these focused on other chemical contaminants such as pesticide residues in food and/or feed, water treatment products or impurities.

3.2. Direct contact with relevant agencies or authorities

A list of worldwide national regulatory agencies and authorities that were contacted is given in Supplementary Table 3. Direct contact did not result in any additional guidance or assessment frameworks to those identified in Table 1.

There were various documents or replies received from contact with

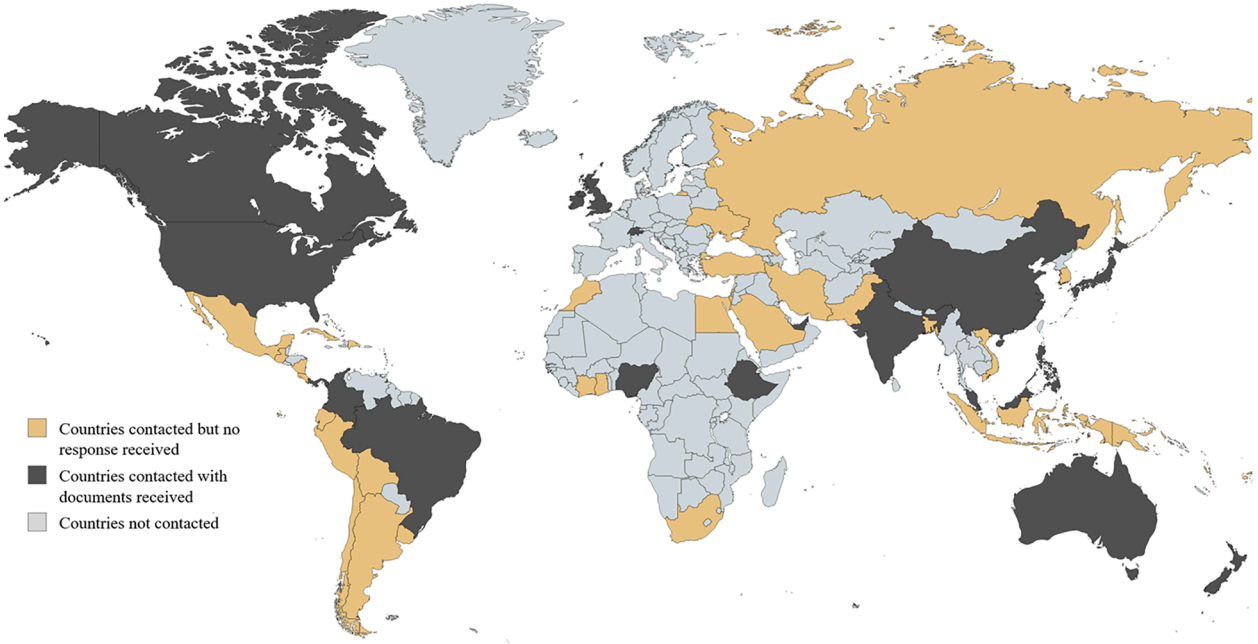

Fig. 2. Countries whose regulatory authorities were identified and contacted.

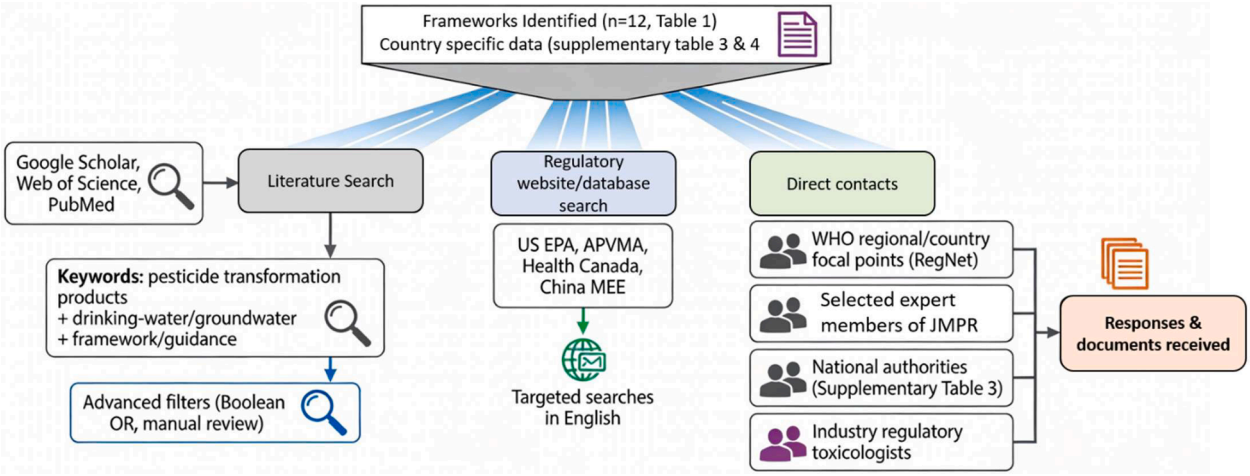

Fig. 3. Methodology for identifying Assessment Frameworks and gathering information.

the WHO focal points, JMPR consulting experts and industry (Supplementary Table 4). In an initial screening, essential information found in the received documents and responses was identified and highlighted to provide a summary of the main points. The summary provides a structured overview of the collective feedback and data gathered from these diverse sources.

Responses were received from various regulatory experts from industry. Again, SANCO/221/2000 and the recent EFSA/ECHA guidance document were identified as the only current guidance documents specifically for assessment of pesticides transformation products in water (Fig. 4).

3.3. Relevant published literature

A critical review of published literature using the search terms described in Section 2 yielded numerous studies that addressed various risk assessment processes and health impacts of pesticide transformation products. Supplementary Table 5 provides a summary of these publications including the relevant chemical/s and/or transformation product/s under assessment (e.g., active substances, metabolites, residues, degradates, etc.), the primary health focus, i.e., human or environmental, as well as the methodology employed, and the availability of toxicity data available or needed for the assessment.

A number of publications did review current assessment frameworks, in particular SANCO/221/2000, and made recommendations for changing the assessment procedure. Several publications also proposed assessment schemes, e.g. for metabolites (Pelkonen et al., 2023); transformation products (Melching-Kollmuß et al., 2010) and water treatment products (Michel et al., 2022). These were reviewed and some important aspects related to the development of a toxicological assessment framework for pesticide transformation products in drinking-water are discussed here. These include, alternative methods to animal testing, the application of the TTC concept, concentration thresholds, other methods for risk assessment, and aggregate exposure.

3.4. Strengths and limitations of existing toxicological assessment frameworks

As well as SANCO/2000/221, which is specifically for groundwater and the recent EFSA/ECHA guidance document, a number of additional frameworks were identified that we also considered potentially relevant for the assessment of pesticide transformation products in the drinking-water context. Each of these documents was considered critically using a list of 32 assessment elements (see supplemental excel file "Evaluation of metabolite assessment schemes.xlsx"). A summary of the strengths and limitations of the frameworks deemed relevant is given in Table 1. An additional, more detailed summary and analysis, particularly of SANCO/221/2000, but also the recent EFSA/ECHA guidance document are provided in Sections 3.4.1 and 3.4.2.

3.4.1. SANCO/221/2000 framework to assess toxicological relevance of pesticide transformation products in groundwater

The SANCO/221/2000 guidance document was first published in 2000. It has since been revised 11 times, the last of which was in October 2021. As a consequence, considerable experience has been garnered with this guidance document.

SANCO/221/2000 provides a comprehensive and structured framework for assessing the toxicological relevance of "metabolites" i.e., transformation products in groundwater. A *relevant metabolite is defined as having "comparable intrinsic properties as the active substance in terms of its biological target activity, or that it has certain toxicological properties that are considered severe and unacceptable with regard to the decision-making criteria described in the text"*, the text referring to the SANCO/221/2000 evaluation of the potential risk (below). A non-relevant metabolite is a *"metabolite which does not meet the criteria provided for "relevant metabolites""*.

SANCO/221/2000 outlines a tiered, stepwise approach to assess the potential risk of transformation products formed from active substances used in plant protection products in groundwater. The assessment involves a five-step approach, including exclusion of non-concerning degradation products, quantification of potential groundwater contamination, hazard assessment (biological activity, genotoxicity, and systemic toxicity, in particular acute toxicity, carcinogenicity, reproductive and developmental toxicity), exposure assessment using a TTC approach derived from the US EPA's threshold of regulation, and refined risk assessments for non-relevant transformation products which is aimed at preventing any unacceptable risks associated with these transformation products even if they are assessed to be non-relevant. The guidance emphasizes maintaining a high level of groundwater protection, ensuring that relevant transformation products do not exceed the permissible concentration limit of 0.1 μg/L and providing a systematic and scientific basis for regulatory decision-making.

The assessment approach uses various screening methods for biological activity and toxicity, and considers both existing data and prescribed additional testing if necessary. It incorporates principles of precaution and aims to harmonize assessment schemes across EU Member States, while also providing flexibility for national authorities to apply the guidelines to their specific contexts. However, this has led to divergent assessments, particularly of non-relevant metabolites (more below). The inclusion of expert judgement, structure-activity relationships (SAR), and TTC helps manage data-poor substances and refine the risk assessment process, ensuring a pragmatic and scientifically valid

Table 1
Strengths and limitations of selected assessment frameworks considered relevant to the drinking-water context. (see reference in table EC, 2003ECHA and EFSA, 2023US EPA, 2013US EPA, 2020WHO, 2015WHO, 2016Japan FSC, 20172024US EPA, 2003US EPA, 2011ICH, 2013EC, 2012OECD, 2009European Commission, 2003EFSA and ECHA, 2023US EPA, 2013US EPA, 2020WHO, 2015WHO, 2016Food Safety Commision, 20172024US EPA, 2003US EPA, 2011ICH, 2013European Commission, 2012OECD, 2009).

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
	Title	Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater (European Commission, 2003)	Impact of Water Treatment Processes on Residues of Active Substances or their Metabolites in Water Abstracted for the Production of Drinking-water (EFSA et al., 2023)	Guidance for Residues of Concern in Drinking-water (US EPA, 2013)	Framework for Conducting Pesticide Drinking-water Assessments for Surface Water (US EPA, 2020)	Guidance Document for WHO Monographers and Reviewers (WHO, 2015)	Module II. Scientific Guidelines for the Preparation of Veterinary Drug Residue Monographs, Working Papers (WHO, 2016)	Guide for Considerations on Residue Definitions for Dietary Risk Assessments of Pesticide Residues (Food Safety Commision, 2017, 2024)	Criteria for Inclusion of Pesticide Metabolites and Degradates in Risk Assessments and Tolerance Expressions (MARC) (US EPA, 2003)	Guidance on Incorporation of Water Treatment Effects on Pesticide Removal and Transformations in Drinking-water Exposure Assessments (US EPA, 2011)	M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals (ICH, 2013)	SANCO 10597 Guidance Document on the Assessment of the Equivalence of Technical Materials of Substances Regulated under Regulation (EC) No 1107/2009 (European Commission, 2012)	Guidance Document on the Definition of Residue (OECD, 2009)
	Purpose	A five-step tiered framework for evaluating or assessing the toxicological so-called relevance of pesticide metabolites in groundwater.	A comprehensive systematic approach for evaluating pesticide transformation products in treated drinking-water.	Guidance on the information and data requirements necessary for assessing the toxicological risks or concerns associated with transformation products and pesticide	A tiered framework for predicting pesticide concentrations in drinking-water, begining with a conservative screening and advancing to a more	A step-wise, comprehensive methodology for assessing the toxicological relevance of pesticides and their residues for calculating dietary exposure	A step-wise, comprehensive methodology for assessing the toxicological relevance of veterinary medicines and their residues for calculating	A four-step methodology for assessing the toxicological relevance of pesticides and their residues for calculating dietary exposure and establishing MRLs.	A structured framework for evaluating pesticide metabolites and degradates in dietary risk assessments which may include	A systematic methodology for assessing the effects of drinking-water treatment procedures on pesticide removal and	A structured framework for evaluating mutagenic impurities in pharmaceuticals.	A tiered framework for evaluating the technical equivalence of pesticide active substances, focusing on impurities and their toxicological relevance.	Harmonized approach for residue definition in dietary risk assessment and MRL enforcement. Differentiates between risk assessment (includes toxicologically significant metabolites/de

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
				residues in drinking-water.	refined assessment utilising models and monitoring data.	and establishing MRLs..	dietary exposure and establishing MRLs.		drinking-water.	transformation.			gradates based on exposure and toxicity) and MRL enforcement (prioritizes a single "marker compound" for practicality).
Strength	**Approach**	Provides a detailed and structured, step-wise, tiered approach for assessing metabolite relevance in groundwater.	Provides a thorough, step-wise, tiered approach for evaluating water treatment transformation products in drinking-water.	Provides a detailed and structured approach for identifying pesticide residues, degradates and transformation products of concern in drinking-water.	Provides a clear, thorough, step-wise, four-tiered approach for predicting pesticides in drinking-water.	Provides a clear, detailed, step-wise assessment scheme that ensures thorough evaluation of dietary residue toxicity.	Provides a clear, detailed, step-wise assessment scheme that ensures thorough evaluation of dietary residue toxicity.	Provides a clear, detailed, step-wise assessment scheme that ensures thorough evaluation of dietary residue toxicity.	Provides detailed toxicological consideration for including metabolites in risk assessments, ensuring thorough evaluation.	Provides a framework for assessing the impact of water treatment processes on pesticide levels in drinking-water, in particular removal of pesticides and their metabolites.	Provides a clear, detailed, step-wise, tiered approach for assessing mutagenic potential of impurities.	Provides a clear, step-wise, tiered approach with a flow-chart for the toxicological assessment of impurities.	Provides toxicological considerations and a clear step-wise approach for defining pesticide residues for both dietary risk assessment and MRL/tolerance enforcement.

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
Limitation	**Complexity and technical expertise**	Can be complex and may require significant resources, requiring sophisticated testing and modelling, potentially limiting its applicability in resource-constrained settings.	Can be complex, requiring significant resources and expertise to implement fully, potentially limiting its applicability in resource-constrained settings.	Complex, requiring significant resources and expertise to implement fully, potentially limiting its applicability in resource-constrained settings.	Complex, requiring significant resources, requiring sophisticated testing and modelling, potentially limiting its applicability in resource-constrained settings.	Can be complex, requiring significant resources, potentially limiting its applicability in resource-constrained settings.	Can be complex, requiring significant resources, potentially limiting its applicability in resource-constrained settings.	Explicit data requirements or guidelines on the types of toxicity data and information required are not provided, potentially leading to inconsistent assessments.	Complex, requiring significant resources, sophisticated testing and modelling, potentially limiting its applicability in resource-constrained settings.	Guidance applies to pesticide active substances only. Requires expertise in water treatment engineering, pesticide chemistry, and transformation pathways.	Complex, requiring significant resources, sophisticated testing and modelling, potentially limiting its applicability in resource-constrained settings.	Can be resource-intensive, potentially limiting its applicability in resource-constrained settings.	Complex, requiring significant resources, potentially limiting its applicability in resource-constrained settings.

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Table 1 (*continued*)

Strength	**Methodological acceptance**	Enhances consistency of assessment by using well-defined criteria for assessing the relevance of pesticide metabolites. Internationally harmonized for EU regulatory use.	Uses well-established scientific principles, incorporating toxicological data and risk assessment methodologies widely recognized in the scientific community. Provisions for incorporating more data as it becomes available. Internationally harmonized for EU regulatory use.	Emphasizes the importance of clear communication and documentation, aiding in transparency and regulatory compliance.	Specific guidance is provided for addressing data gaps in predictive models, Pesticide in Water Calculator (PWC) and the Pesticide in Flooded Application Model (PFAM).	Uses well-established scientific principles, incorporating toxicological data and risk assessment methodologies widely recognized in the scientific community Internationally harmonized under FAO/WHO and aligned with Codex Alimentarius and VICH guidelines.	Uses well-established scientific principles, incorporating toxicological data and risk assessment methodologies widely recognized in the scientific community Internationally harmonized under FAO/WHO and aligned with Codex Alimentarius and VICH guidelines.	Ensures consistency with international risk assessment methodologies, promoting transparency.	Relies on a robust scientific basis, namely extensive (toxicological) databases.	Emphasis on detailed data collection, including physicochemical properties and environmental fate, ensures accurate modelling and risk assessment.	Uses well-established scientific principles, incorporating toxicological data and risk assessment methodologies widely recognized in the scientific communit.y Internationally harmonized (ICH) and adopted by FDA, EMA, and other major regulatory bodies.	Uses well-established scientific principles, incorporating toxicological data and risk assessment methodologies widely recognized in the scientific community. Includes detailed guidance on evaluating toxicity data, establishing ADI with a detailed approach for quantifying exposure using models and monitoring data. Internationally harmonized for EU regulatory use.	Uses well-established scientific principles, incorporating toxicological data and risk assessment methodologies widely recognized in the scientific community. Draws on internationally harmonized principles (e.g., Codex) and is widely recognized among OECD member countries. Emphasizes flexibility and scientific judgment.

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
Limitation	**Methodology**	Reliance on expert judgment, can introduce variability in assessments. Lacks detailed guidance concerning the evaluation of so-called convincing evidence and for specific analytical methods for detecting and quantifying metabolites in groundwater.	While it provides approaches for data-poor substances, the lack of concrete data can still lead to significant uncertainty in the risk assessment outcomes. Reliance on expert judgment, can introduce variability in assessments..	Reliance on expert judgment, particularly the interpretation of environmental fate and toxicity data, can introduce variability in assessments.	The models and assumptions used in the guidance may not be easily adaptable to diverse environmental conditions found in different regions, such as varying climates, soil types, and agricultural practices.	The detailed nature of the guidelines may lead to variability in implementation across different regions and contexts.	The detailed nature of the guidelines may lead to variability in implementation across different regions and contexts.	Lacks detailed guidance on assessment of hazard and exposure.	Differences in definitions and terms used across international guidelines may cause confusion and inconsistencies.	Laboratory-scale studies, such as jar tests, may overestimate removal efficiencies compared to actual treatment plant performance, leading to potential inaccuracies in risk assessment.	No detailed analytical methods for detecting and quantifying impurities.	Reliance on expert judgment, can introduce variability in assessments.	Reliance on expert judgment, can introduce variability in assessments.

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
Strength	**Data requirements**	Prescribes *in vitro* and *in vivo* testing, ensuring a robust scientific basis.	Data requirements for certain key parameters relating to efate and modelling are clear.	Data requirements for certain key parameters relating to efate and ecotoxicology are clear.	Data requirements primarily for certain key parameters relating to efate and ecotoxicology are clear.	Provides specific methods for assessing toxicity and establishing HBGVs. Considers both parent pesticides and their residues in the assessment process.	Provides specific methods for assessing toxicity and establishing HBGVs. Considers both parent pesticides and their residues in the assessment process.	Considers both parent pesticides and their residues in the assessment process. Utilizes a wide range of toxicity studies and expert judgment to establish HBGVs.	Requires comprehensive metabolism and efate data for parent and metabolites/ degradates	Requires data on pesticide physicochemical properties, treatment process efficacy.	Provisions for genotoxic impurities with thresholds.	The scheme considers the inherent toxicity and toxicity of impurities relative to the parent compound.	Provides specific considerations for assessing toxicity and establishing HBGVs. Considers both parent pesticides and their residues in the assessment process.

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
Limitation	**Data-requirements**	Extensive data requirements for a thorough assessment may be resource-intensive and costly. *In vitro* and potentially also *in vivo* data are required for any metabolite predicted to be in groundwater > 0.1 µg/L.	Extensive data requirements for a thorough assessment may be resource-intensive and costly. *In vitro* and potentially also *in vivo* data are required for treatment products at higher concentrations.	Does not provide detailed data requirements for the toxicological assessment.	Does not provide any data requirements for the toxicological assessment.	Relies on comprehensive dossiers with a substantial amount of data and information. Assessment is resource-intensive.	Relies on comprehensive dossiers with a substantial amount of data and information. Assessment is resource-intensive.	Primarily targets biological metabolites, with no emphasis on environmental transformation products.	Relies heavily on available data, which may be insufficient for certain metabolites, potentially leading to underestimation of risks.	Requires extensive data collection and analysis.	Does not provide detailed, prescriptive data requirements for toxicological assessment, but relies on available data	Requirement for sophisticated approaches and additional toxicity data in Tier II may be resource-intensive and costly. *In vitro* and potentially also *in vivo* data are required for Tier II or higher assessments.	Does not provide explicit data requirements for toxicological testing. Relies on expert judgement.

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
Strength	**Data-poor substances**	Includes pragmatic approaches like the threshold of concern for data-poor substances, facilitating practical implementation	Utilizes predictive models (like (Q)SAR), databases and read-across approaches for assessing the behaviour and toxicity of transformation products, as well as TTC for data-poor substances.	Utilizes predictive models (e.g. ECOSAR and (Q)SAR) approaches for assessing the behaviour and toxicity pesticide residues, degradates and transformation products.	Conservative assumptions and uncertainty factors are proposed *in lieu* of some data. Estimated pesticide concentrations from the DWA are incoporated into an aggregated human health risk with consideration of various population (sub-) groups and exposure durations.	Utilizes predictive models (like (Q)SAR), databases and read-across approaches for assessing the behaviour and toxicity of transformation products, as well as TTC for data-poor substances.	Utilizes predictive models (like (Q)SAR), databases and read-across approaches for assessing the behaviour and toxicity of transformation products, as well as TTC for data-poor substances.	Encourages comprehensive use of all available scientific data, including toxicology databases and published studies; allows reference to international evaluations and weight-of-evidence approaches when direct data are limited.	Includes provisions for data-poor compounds and theoretical metabolites, allowing for comprehensive assessments even with limited data. Basic considerations for aggregate exposure, i.e. dietary risk assessment may include exposure via drinking-water in addition to food.	Allows use of analogies (e.g., organophosphate data for similar compounds) and theoretical properties (e.g., hydrolysis rates) when empirical data are lacking.	Utilizes predictive models (like (Q)SAR), databases and read-across approaches for assessing the behaviour and toxicity of impurities present at lower amounts.	Utilizes predictive models (like (Q)SAR), databases and read-across approaches for assessing the behaviour and toxicity of impurities present at lower amounts.	Allows for the use of available monitoring data and theoretical considerations for metabolites/degradates not observed in studies. Residue definitions may be based on the parent compound when little is known about metabolites

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
Limitation	**Data-poor substances**	No clear guidance is given for further evaluating non-relevant metabolites, particularly at concentrations above 10 µg/L	Reliance on predictive methods like (Q)SAR and read-across may introduce uncertainties when empirical data are lacking.	Reliance on predictive methods like (Q)SAR and read-across may introduce uncertainties when empirical data are lacking.	Higher-tier assessments require more sophisticated approaches and data, which may not be readily available, particularly in resource-constrained settings. While the framework is robust, there may be limited specific guidance on assessing newly emerging contaminants that were data-constrained at the time of the document's creation.	Reliance on predictive methods like (Q)SAR and read-across may introduce uncertainties when empirical data are lacking.	Reliance on predictive methods like (Q)SAR and read-across may introduce uncertainties when empirical data are lacking.	Does not provide detailed provisions for assessing data-poor compounds, i.e. (Q)SAR and read-across approaches are not clearly integrated into the assessment framework and TTC is not explicitly mentioned.	No detailed provisions for (Q)SAR or read-across.	Does not provide detailed provisions for assessing data-poor compounds (Q)SAR and read-across approaches are not clearly integrated into the assessment framework.	Reliance on predictive methods like (Q)SAR and read-across may introduce uncertainties when empirical data are lacking.	Reliance on predictive methods like (Q)SAR and read-across may introduce uncertainties when empirical data are lacking.	The document does not provide detailed provisions for predictive (Q)SAR or read-across approaches

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Table 1 (*continued*)

Strength	**Exposure**	Quantitative approach with modelling, monitoring, and scenario analysis	Evaluates the effectiveness of various water treatment processes and their impact on the formation and removal of metabolites. Exposure assessment is important to estimate the total intake of TP (in Tier 2).	Provisions are made for data and information pertinent to the effects of water treatment processes.	Detailed methodologies for quantifying exposure, incorporating both modelling and real-world monitoring data. Potential effects of water treatment with the example of organophosphate are considered. Estimated pesticide concentrations are incoporated into an aggregated human health risk with consideration of various population (sub-) groups and exposure durations.	Includes comprehensive approaches for dietary exposure calculations and MRL establishment while addressing the needs of various population (sub-) groups if needed. Consideration is given to aggregate exposure.	Includes comprehensive approaches for dietary exposure calculations and MRL establishment while addressing the needs of various population (sub-) groups if needed. Structured methods for aggregate exposure.	Exposure assessment is central to residue definition for risk assessment. The guidance requires a comprehensive, multi-source approach using metabolism and residue studies from plant, animal, and fish products, and includes metabolites/degradates	Separate consideration of metabolites and environmental degradates. Basic considerations for aggregate exposure, i.e. dietary risk assessment may include exposure via drinking-water in addition to food	Highlights the potential formation of toxic transformation products and ensures a more complete evaluation of health risks.	Provides clear methodologies for quantifying (less-than-lifetime, i.e. short-term) human exposure in combination with cumulative effects.	Exposure calculation is clearly defined, supporting harmonized and transparent decisions.	Considers both the potential for exposure and relative toxicity of metabolites/degradate. Exposure assessment is central to residue definition for risk assessment

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
Limitation	**Exposure**	Does not consider aggregate exposure to common metabolites or cumulative exposure.	Although it considers total exposure, there is less emphasis on aggregate exposure from all possible routes, which could lead to underestimation of risks.	Does not consider aggregate exposure to common metabolites or cumulative exposure.	Does not consider aggregate exposure to common metabolites or cumulative exposure.	There is a minimal consideration on aggregate exposure but no mentioned of cumulative exposure. Focuses on individual compounds or defined groups relevant to the parent pesticide.	Does not consider aggregate exposure to common metabolites or cumulative exposure. Focuses on individual compounds or defined groups relevant to the parent pesticide.	Does not consider aggregate exposure to common metabolites or cumulative exposure. Focuses on individual compounds or defined groups relevant to the parent pesticide.	Does not explicetly address aggregate/cumulative exposure	Limited availability of comprehensive monitoring data on pesticides and metabolites in finished drinking-water can hinder accurate assessment.	Primarily considers exposure in the general population, with less emphasis on other sensitive populations.	Focus on individual impurities and does not consider cumulativeor aggregate exposure.	Does not address aggregate or cumulative exposure from multiple residues or sources directly. Focuses on individual compounds or defined groups relevant to the parent pesticide.

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
	Refinement	In theory, monitoring information can be used to reine model predictive models.	Encourages iterative refinement using new data (e.g., updated monitoring data)	Utilizes monitoring data at higher tiers ensuring accurate detection and assessment of residues and transformation products.	Allows for spatial and temporal refinements in the assessment process, enabling the evaluation of pesticide impacts in specific regions and under varying environmental conditions. Monitoring information feeds into consideration of the duration of the exposure.	Supports iterative updates, use of monitoring data, and refinement as new data emerge	Encourages iterative updates with new data.	Includes approaches for evaluating genotoxicity, particularly *in vivo* studies.	Encourages iterative updates as new data emerge; improved methods can prompt reassessment	Consideration of local water treatment practices and variability in process efficacy enhances the realism and applicability of exposure assessments. Clear guidelines for laboratory and full-scale studies provide a structured approach to data collection and evaluation.	Refinement of exposure assessment is possible if data are available.	Allows for refinement with new data and expert judgment	Encourages refinement of residue definitions as new data become available.

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Table 1 (*continued*)

		EC, 2003	ECHA and EFSA, 2023	US EPA, 2013	US EPA, 2020	WHO, 2015 (JMPR)	WHO, 2016 (JECFA)	Japan FSC, 2017 & 2024	US EPA, 2003	US EPA, 2011	ICH, 2013 (US FDA)	EC, 2012	OECD, 2009
	Water treatment processes	Does not address the impact of water treatment processes on metabolite degradation.	Assesses the impact of major drinking-water treatment processes. Evaluates the formation and toxicological relevance of transformation products generated during these processes.	Does not address the impact of water treatment processes on metabolite degradation. It does not explicitly emphasize the use of human data such as epidemiological studies, which could enhance risk assessments.	Considers the impact of standard water treatment processes (e.g., sedimentation, flocculation, chlorination, activated carbon filtration) on pesticide residues where data exist	Does not systematically address water treatment processes or their effects on residues/metabolites	Not addressed as it focuses mostly on veterinary drug residues in food	Does not address the impact of water treatment processes on residue degradation.	Minor metabolites with significant toxicological concerns might be overlooked due to the primary focus on major metabolites.	Assessment scheme for toxic potential of transformation products and/or residues of concern is not provided.	Is limited to an assessment of genotoxic potential of impurities in pharmaceuticals.	Reliance on predictive methods like SAR and (Q)SAR may introduce uncertainties when empirical data are lacking.	Does not provide guidance on the effects of water treatment processes on residues in drinking-water

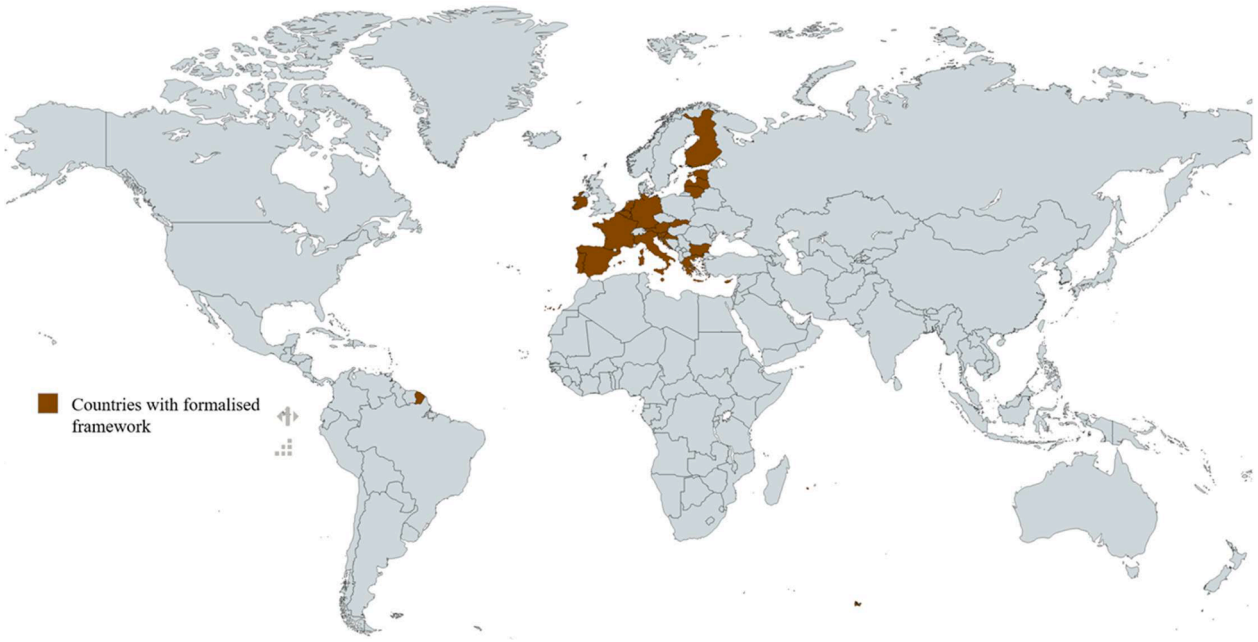

Fig. 4. Countries with formalised framework specific to Pesticide transformation products in water.

approach to protecting groundwater resources from pesticide contamination.

Although the aim of SANCO/221/2000 is to harmonise the risk assessment of pesticide transformation products, 24 years of use within the EU has clearly showen that MSs interpret and apply SANCO/221/2000 differently.

i) Exemplary of this is the hazard assessment in Stage 2 of Step 3, where transformation products are assessed for their genotoxic potential, and the conclusions can differ between MSs for various reasons:
   - To address genotoxic potential, gene mutation (in bacteria and mammalian cells), clastogenicity and aneugenicity "*must be assessed*" using "*a combination of an Ames test, an in vitro Mammalian Cell Gene Mutation Test (*tk *or* hprt *locus) and an in vitro micronucleus test*". Nonetheless and as explicitly stated on page one, SANCO/221/2000 "*does not intend to produce legally binding effects*". Consequently, member states may have differing views on the acceptance of (Q)SAR analyses as a surrogate for these endpoints;
   - As science has progressed, some OECD Test Guidelines (TGs), such as the *in vitro/in vivo* chromosomal aberration test (CA), the *in vitro/in vivo* unscheduled DNA synthesis (UDS), and the *in vivo* comet assay, are no longer considered appropriate for addressing certain endpoints (OECD, 2022);
   - Changes to the criteria for the evaluation and interpretation of results in the OECD TGs have led to situations where results that were previously considered clearly negative are now viewed as equivocal or, in very rare cases, positive, or vice versa (ICH Harmonised Tripartite, 2011; OECD, 2022);
   - Member states may interpret the same results of these OECD tests differently based on differing expert opinions;
   - What constitutes evidence of bone marrow exposure (in relation to the *in vivo* micronucleus test) has evolved over time (ICH Harmonised Tripartite, 2011; OECD, 2016).

ii) Different interpretations can also occur at other steps of the assessment process. For example, the classification of the parent substance in Stage 3 of Step 3 as carcinogenic, reproductive, and/or developmental toxic has direct consequences for its transformation products. These are considered relevant by default unless "convincing evidence" is provided to the contrary, for example through a scientifically sound read-across approach or an appropriate study conducted with the transformation product itself. The level of evidence required to negate relevance varies greatly between member states and is subject to expert judgement.

iii) Similarly, reference values are essential for the refined risk assessment in Step 5. Often, the reference value/s of the parent substance are used for the transformation product. Since the publication of ECHA's Read-Across Assessment Framework (ECHA, 2017a), there has been an increasing requirement for a scientifically founded read-across, which includes a well-defined hypothesis, supporting data, and a robust scientific justification. Again, the approach for and interpretation of these elements can vary between member states according to expert judgement.

iv) While SANCO/221/2000 provides guidance on the application of uncertainty factors, such as recommending an additional 10-fold factor when extrapolating from a 90-day study to chronic exposure, MSs may apply different or additional uncertainty factors (EFSA Scientific Committee, 2012). These decisions often depend on the availability of further substance-specific information including, but not limited to, experimental data and the specific outcomes of a study and also experts may differ on whether information provided is sufficient.

v) The data submitted and evaluated during the approval process for active substances (a process at the EU level managed by the European Commission in coordination with EFSA and ECHA), which includes a representative product formulation, may differ from the data provided in subsequent zonal or national authorisation procedures for plant protection products (a process managed by the MSs in each of the three zones).

vi) As noted above, the primary guidance document for the assessment of pesticide transformation products is SANCO/221/2000, which is grounded in Article 3(32) of Regulation (EC) No 1107/2009. It is specifically focused on the assessment of transformation products as "relevant" or "non-relevant". One significant limitation of this guidance document is that is provides little guidance on the assessment of non-relevant transformation products. This gap has contributed to significant variations in the acceptable concentrations of transformation products across

different EU member states (this is discussed further in Section 4.2.3).

Finally, it should also be noted that SANCO/221/2000 and the FOCUS model are used in the context of the *ex ante* assessment of the plant protection active substance for their approval and for authorisation of plant protection products. The FOCUS (FOrum for the Coordination of pesticide fate models and their USe) model is an essential tool developed by a consortium of European experts to support the risk assessment process for plant protection products. Its primary role is to predict the concentrations of active substances and their transformation products (metabolites) in groundwater that may result from agricultural use of pesticides. The model utilizes a set of standard scenarios that reflect diverse European soil, crop, and climatic conditions representative of typical agricultural practices across the EU. As a consequence, SANCO/221/2000 and the FOCUS model aim to predict the concentration at which a transformation product is potentially present in groundwater above 0.1 μg/L or not, and then assess toxicological relevance.

Some EU national authorities also apply national regulations or a national framework for assessing *ex post* metabolite relevance or manage monitored metabolites above the EU regulatory limit of 0.1 μg/l (see Supplementary Table 4). In most cases, the aim of these national frameworks is to support water monitoring plans and the management of exceedances of regulatory threshold values for pesticide transformation products measured in drinking-water.

3.4.2. EFSA/ECHA guidance document on the impact of water treatment processes on residues of active substances or their metabolites in water abstracted for the production of drinking-water

The EFSA/ECHA guidance document on the impact of water treatment processes on residues of active substances or their metabolites (i.e., transformation products) in water abstracted for the production of drinking-water was published in 2023 and even more recently came into effect. As a consequence, little experience has been gained to date applying the assessment framework to potential water treatment transformation products, although it should be noted that this framework is in principle the same as that proposed by the EFSA for dietary residues in food and feed (EFSA Panel on Plant Protection Products and their Residues (PPR), 2016).

The guidance document provides a comprehensive and structured framework for assessing the formation, presence, and health impacts of pesticide transformation products in treated drinking-water. The document outlines a detailed, step-wise approach for identifying and evaluating transformation products resulting from various water treatment processes such as chlorination, ozonation, filtration and activated carbon adsorption. It emphasizes the importance of understanding the physicochemical properties of both parent compounds and their transformation products, including parameters like water solubility, partition coefficients, and degradation rates. The guidance also stresses the need for robust analytical methods, predictive modelling, and tiered risk assessment strategies to ensure that all potential risks to human health are adequately addressed.

In the assessment process, the guidance includes the consideration of exposure levels, default values for body weight and water intake, and the use of conservative assumptions to manage uncertainties. Toxicity assessments are conducted by comparing the toxicity of metabolites relative to the parent compound and using methods such as read-across, (Q)SAR models, and the TTC approach for data-poor compounds. The document also differentiates between various types of toxicological endpoints, including genotoxicity, carcinogenicity, and systemic toxicity, and proposes specific adjustment factors to account for variabilities in human sensitivity. However, the guidance does not explicitly incorporate epidemiological data, human data, or biomonitoring results, focusing instead on experimental and predictive data sources.

Some key differences in comparison to SANCO/221/2000 are that a) information from rat metabolism (so-called ADME) studies is considered, b) considerably more weight is given to the use of *in silico* analyses, for example, (Q)SAR predictions of Ames bacterial mutagenicity, might be used as a surrogate for an actual Ames test, c) there is no requirement for a third test of mammalian cell mutagenicity and d) relative potency factors are used instead of additional uncertainty factors.

3.4.3. Toxicological assessment frameworks potentially relevant for the drinking-water context

Ten further frameworks (Table 1) were identified as being relevant for the assessment of pesticide transformation products in drinking and groundwater. This selection was based on an initial screening review and the following inclusion criteria: i) the framework is related to pesticide transformation products in water; or, ii) the framework is for dietary residues, or, iii) the framework is for impurities. Frameworks on impurities in chemical products were included, as impurities are often data scarce but structurally related to the main component, as are pesticide transformation products. Following critical consideration of each of the full text documents with 32 detailed review questions (supplementary excel file), a list of strengths and limitations for each was compiled (Table 1). This two-step procedure was applied in analogy to the EFSA Guidance on the Application of systematic review methodology to food and feed safety assessments (EFSA, 2010).

It is also important to note that work is currently underway to update the OECD Guidance Document on the Definition of Residue (OECD, 2009). This update will include a considerably more detailed toxicological evaluation as well as some considerations for pesticide transformation products in drinking-water. This is yet to be finalised and has not yet been published. As a consequence, only the current version of the document that is presently available could be reviewed here.

4. Critical analysis: three fundamental tensions

4.1. Tension 1: hazard classification vs. risk characterization

The EU relies on a primarily hazard-based assessment, whereas most other frameworks are risk-based assessments. SANCO/221/2000 employs a "relevant vs. non-relevant" assessment, whereby a metabolite is deemed relevant if it has comparable intrinsic properties to the parent substance in terms of biological target activity or if it possesses certain toxicological properties considered severe and unacceptable. The binary result of this assessment has immediate regulatory consequences: relevant metabolites are subject to the 0.1 μg/L threshold, while non-relevant metabolites may be permitted at higher concentrations defined by individual Member States.

The relevance assessment offers several advantages. It provides clear regulatory triggers that facilitates enforcement, emphasizes precautionary protection through conservative threshold limits, and benefits from 24 years of implementation. However, this approach has significant limitations in practice and despite the harmonization intent of SANCO/221/2000, Member State interpretations diverge markedly. These issues are discussed in more detail in Section 3.4.1 above, as well as Section 4.2.3 and Table 2 below.

In contrast, frameworks from the US EPA, JMPR, JECFA, and Japan FSC employ risk-based approaches that do not categorize transformation products as relevant or non-relevant. These frameworks conduct an initial genotoxicity screening, assess carcinogenicity, reproductive/developmental and systemic toxicity relative to the parent compound, derive health-based guidance values (HBGVs such as ADI, TDI, or RfD) for quantitative risk characterization. TTC values are employed as a pragmatic tool when specific data are lacking. For instance, the WHO and the US EPA use risk-based approaches and establish guideline values for each pesticide individually and transformation products if sufficient information is available, based on its toxicological properties and potential health risks (see Table 8.1–3 in the GDWQ). This approach involves detailed assessments of each pesticide's health impacts and

Table 2
Threshold values applied by various (former) EU MSs for pesticide transformation products deemed "non-relevant" according to SANCO/221/2000.

Austria	Action values between 0.75 and 10 μg/L for non-relevant transformation products in drinking-water, as defined by the Ministry of Health. Specific action values for certain non-relevant metabolites: 0.3 μg/L, 1.0 μg/L, 3.0 μg/L
Denmark	All pesticide transformation products must meet the 0.1 μg/L limit value in drinking and groundwater.
England and Wales	There is no specific limit provided for non-relevant transformation products; the general 0.1 μg/L limit for pesticides and their relevant transformation products is applied.
France	For raw water used for drinking water abstraction: a limit value of 2 μg/L for individual pesticide active substances and for individual relevant transformation products and 5 μg/L for the sum of all pesticide active substances and relevant transformation products. For finished drinking water: the 0.1 μg/L limit value is applied for individual pesticide active substance and for the individual relevant transformation products and 0,5 μg/L is applied for the sum of all pesticide active substances and relevant transformation products. For each individual non-relevant transformation products, a limit value of 0.9 μg/L is set.
Germany	The German Federal Environment Agency (Umweltbundesamt) has defined two health-related indication values (GOW): 1 μg/L for non-relevant transformation products where toxicological data is available. 3 μg/L as an upper limit for non-relevant transformation products, again depending on available toxicological data. An upper precautionary action value (Vorsorgemaßnahmenwert, VMW) of 10 μg/L is allowed temporarily for a maximum of 10 years at a specific site.
Netherlands	A limit value of 1.0 μg/L for non-relevant transformation products is established for tap water, but the ADI of the parent can be used for non-relevant transformation products present at >1 ug/L in drinking-water sources, although a lower ADI for the transformation product may be derived if required and toxicological evidence is available.
Switzerland [a]	2 μg/L based on a modified TTC III value with an additional UF of 3 for infants [b].

[a] Value and information provided following direct contact with relevant agency (Section 3.2).
[b] EFSA Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee et al., 2019).

calculating acceptable exposure levels. Consequently, WHO guideline values for pesticides can vary widely depending on the pesticide's specific characteristics, sometimes being higher or lower than 0.1 μg/L, depending on the toxic potential.

This fundamental difference has practical implications for regulatory outcomes. The EU's primarily hazard-based approach may result in stringent limits for compounds of low toxicological concern, whereas the risk-based approach requires sufficient data, often unavailable for transformation products, to establish HBGVs, which can hinder regulatory decision making when data are insufficient.

4.2. Tension 2: data-rich vs. data-poor scenarios

As noted, the assessment of pesticide transformation products frequently encounters a fundamental practical challenge, many transformation products only very rarely have a complete toxicological data set comparable to parent active substances. This data-poor substance scenario necessitates pragmatic approaches that balance protective decision-making with resource constraints. To this end, new approach methodologies and TTC offer some potential solutions.

4.2.1. New approach methodologies

New approach methodologies (NAMs), especially *in silico* methods have gained increasing regulatory acceptance, particularly with regards to genotoxicity testing (Feigenbaum et al., 2015; Pelkonen et al., 2023) but also for ecotoxicological endpoints (Chang et al., 2020). Currently, (Q)SAR models of Ames mutagenicity are considered sufficiently reliable and predictive as to be a viable surrogate for actual testing and (Q)SAR predictions of Ames mutagenicity are now used in several regulatory areas (Feigenbaum and Worth, 2019; Reilly et al., 2019). For instance, the ICH M7 guideline allows for the use of (Q)SAR models for the assessment of mutagenic impurities in pharmaceuticals. According to this guideline, (Q)SAR predictions can be used instead of *in vitro/vivo* toxicological testing for the initial assessment of impurities (ICH, 2013). The reliability of (Q)SAR predictions for other endpoints, e.g., clastogenicity and aneugenicity, is, however, lacking and current practice is to use (Q)SAR models as a basis for a read across from tested substances (EFSA, 2020; EFSA and ECHA, 2023; Fischer et al., 2024). While models exist for higher-tier endpoints, their regulatory acceptance is generally more limited due to the complexities of modelling higher tier endpoints.

The tiered approach to toxicological assessment employed in SANCO/221/2000 has provisions for the use of NAMs. (Q)SAR predictions and read-across approach are standard practice although there are still notable limitations, particularly concerning the higher-tier endpoints. Assessment varies considerably across jurisdictions, particularly for non-genotoxic endpoints, meaning that identical substances be assessed differently by different MSs.

Various reviews were also identified which discuss the use of (Q)SARs combined with TTC values (Czaja et al., 2020), *in vitro* testing (Pelkonen et al., 2023), NAMs (Stucki et al., 2022) and provide some alternatives to carcinogenicity and developmental toxicity testing (Corvi et al., 2019). Although regulatory acceptance proves to be a considerable hurdle, for many of these newer NAMs (Schmeisser et al., 2023; Sewell et al., 2024; Van Der Zalm et al., 2022), continuing efforts and progress are being made with respect to acceptance (Kienhuis et al., 2025; Marx-Stoelting et al., 2023; Utrecht University, 2023).

4.2.2. Application of the TTC concept

A comprehensive review of the TTC concept and its use in the context of pesticide transformation products in drinking water is beyond the scope of this review, and several such commendable reviews already exist (Boobis et al., 2017; Feigenbaum et al., 2015; Gimsing et al., 2019; Hartung, 2017). However, due to its importance, a brief synopsis is provided here, along with considerations for a proposed assessment framework (see Sections 4 and 5 below).

The TTC concept was originally introduced as the Threshold of Regulation (TOR) approach for indirect food additives by the US EPA. Since then, it has been adopted for use in other areas, including cosmetics, industrial chemicals, pharmaceutical impurities, food contact materials, pesticides, their residues, and transformation products (Hartung, 2017).

Initially derived from the Carcinogenic Potency Database, the TOR was set at 0.5 ppb in the diet, corresponding to 1.5 μg/person per day or 0.025 μg/kg body weight per day (Cheeseman et al., 1999). This threshold was adopted by SANCO/221/2000 and is the applicable concentration subsequent to the hazard assessment in Step 3.

Relevant structural information according to (Cramer et al., 1976), was derived from 613 chemicals (including industrial, food, environmental, agricultural, pharmaceutical, and consumer product chemicals) with 2941 NOAELs to develop three different TTC values (Munro et al., 1996). These values were further refined to include specific thresholds for organophosphates and carbamates, as well as DNA-reactive mutagens (Kroes et al., 2004).

Several studies have subsequently validated the TTC values using pesticides and pesticide transformation products (Chang et al., 2020; Cramer et al., 1976; EFSA, 2020). Other studies have argued that these values are also sufficiently protective for other toxicological endpoints, notably developmental and reproductive toxicity (Bernauer et al., 2008; Laufersweiler et al., 2012; Van Ravenzwaay et al., 2011) and repeated dose toxicity (Tluczkiewicz et al., 2011). Additionally, a TTC approach has been proposed for potential use in ecotoxicological risk assessment (Jiang et al., 2023).

Mons et al. (2013) applied the TTC concept to assess the relevance of

pesticide transformation products in drinking-water, proposing TTC as a pragmatic tool for the initial screening of chemicals without extensive toxicity data. Similarly, Feigenbaum et al. (2015) concluded that the TTC concept is sufficiently protective for the majority of cases and proposed additional values for transformation products deemed "non-relevant" according to SANCO/221/2000 (Magurany et al., 2023).

4.2.3. Concentration thresholds and member state divergence

As mentioned above, fixed concentration thresholds of 0.1 and 0.5 μg/L have been established as acceptable limits for pesticides and the sum of pesticides and "relevant" transformation products in drinking-water in the EU Drinking-water Directive (2020/2184) (European Parliament and Council of the European Union, 2020) and Groundwater Protection Directive (2006/118/EC) (European Union, 2006) . The basis for these thresholds was not toxicological, rather the analytical capabilities at the time the directive was initially written and applying the precautionary principle, a central tenet of EU environmental policy. This limit was subsequently adopted as the threshold for "relevance" in SANCO/221/2000. According to the responses received by regulators and industry (Section 2.2 above and documents in Supplementary Table 4), 0.1 μg/L and 0.5 μg/L appear to have been widely adopted by many non-EU countries as well.

SANCO/221/2000 also employs a second threshold concentration of 0.75 μg/L, based on the TOR, but no specific thresholds exist for "non-relevant" transformation products. Consequently, national authorities are left to determine acceptable concentrations themselves, rather than applying a harmonized threshold. This has resulted in thresholds varying from 0.1 μg up to and in some specific cases beyond 10 μg/L (Table 2) demonstrating a key limitation of the relevance assessment approach.

Alternative threshold concentrations have been proposed, for example, a general safe limit value of 4.5 μg/L was proposed by Laabs et al. (2015) for "non-relevant" transformation products as assessed using SANCO/221/2000. This value was derived from the TTC Cramer Class III value of 90 μg/kg bw per day, divided by 2 L water consumption per day and applying the relative allocation factor of 10 %. A threshold of 3.0 μg/L in drinking-water was also proposed Melching-Kollmuß et al. (2010), based on the same water consumption and relative allocation factor, but using a more conservative threshold value of 60 μg/person per day to ensure coverage of specific toxicological concerns such as reproductive and developmental toxicity. This value was considered by the authors to be a safe level below which no further toxicological testing is required.

4.3. Tension 3: environmental TPs vs. water treatment TPs

A critical and inadequately addressed gap in most frameworks concerns transformation products formed during water treatment processes. Of the frameworks identified, only the EFSA/ECHA guidance (2023) specifically addresses water treatment transformation products. SANCO/221/2000 focuses exclusively on environmental degradates, the US EPA (2011, 2020) documents acknowledge water treatment effects without providing structured assessment guidance, and JMPR, JECFA, and Japan FSC guidance do not address water treatment transformation products at all. This represents a significant blind spot in global drinking water protection.

The organophosphate chlorination example illustrates this concern. Chlorination of water containing organophosphate pesticides generates oxons, sulfoxides, sulfones, and/or oxon-sulfones—transformation products substantially more potent than the parent compounds (Fig. 1). This is not an isolated example. Chlorination can oxidize thiophosphates to more potent oxons and form novel chlorinated byproducts, ozonation generates oxidation products with largely unknown toxicity profiles, UV treatment causes photodegradation to novel structures with uncertain hazard profiles, and activated carbon selectively removes parent compounds while allowing certain transformation products to pass through. A regulatory paradox emerges that is current frameworks may approve a pesticide based on environmental degradate profiles, yet the actual human exposure in finished drinking water may be dominated by treatment-generated transformation products never formally evaluated.

The EFSA/ECHA guidance (2023) represents significant progress, requiring identification of potential water treatment transformation products, evaluation of formation kinetics under relevant conditions, and toxicological assessment using the same tiered approach applied to dietary residues (EFSA Panel on Plant Protection Products and their Residues (PPR), 2016). However, this guidance has only recently come into effect, and implementation experience is limited. Its fundamental approach of integrating treatment transformation product assessment into the formal framework could serve as a model for global guidance, though adaptation for resource-constrained settings would be necessary. Without such explicit inclusion in all major frameworks, water treatment-derived transformation products will remain inconsistently assessed across jurisdictions.

5. Aggregate and cumulative exposure: A neglected dimension

An important limitation for most of the existing frameworks is the inadequate consideration of aggregate and cumulative exposure to pesticide transformation products. In the GDWQ, aggregate exposure to non-DNA reactive chemicals is accounted for using a relative source allocation factor, a percentage of the ADI allocated to exposure via drinking-water (see Section 8.2.2 of the GDWQ). The default is 10–20 % of the ADI, but if sufficient information is available concerning dietary exposure, this may be adjusted up to a maximum of 80 %. This practice has been adopted widely, for example by some EU member states and Japan.

In a comprehensive review, (Rotter et al., 2018) cite several step-wise frameworks for risk assessment of mixtures, namely; The WHO/IPCS's tiered framework for the risk assessment of combined exposure to multiple chemicals; EFSA's flexible, overarching framework for human, animal, and ecological mixture risk assessment (EFSA, 2008); The US EPA's decision trees and tiered processes for cumulative risk assessment (US EPA, 2000, 2008); The US Agency for Toxic Substances and Disease Registry's framework specifically for assessing chemical mixtures at sites of environmental contamination (Agency for Toxic Substances and Disease Registry, United States, 2004) and the Scientific Steering Committee of the Norwegian Scientific Committee for Food Safety's step-by-step approach for the risk assessment of multiple chemical exposures. Health Canada also have published some principles for assessing aggregate exposure, including drinking-water and food (Health Canada, 2003) and ECHA has a tiered approach to combined exposure assessment in Section 4.4.1 of Volume III of their Guidance for Biocidal Products Regulation (ECHA, 2017b). These frameworks share common features, such as the use of tiered approaches, decision trees, and a focus on both exposure and hazard data.

Of particular relevance within the context of the GDWQ is the International Programme on Chemical Safety's (IPCS) tiered framework for the risk assessment of combined exposure to multiple chemicals (Meek et al., 2011). Indeed, this framework was adapted for the drinking-water context in the publication *Chemical mixtures in source water and drinking-water* (WHO, 2017). Like other approaches above, this approach may be increasingly refined as the amount of data increases. Tier 0 is a simple, semi-quantitative estimation of aggregate exposure and risk using conservative assumptions to identify whether there is potential concern. If the assessment shows an adequate margin of exposure (MoE), no further analysis is needed. Tier 1 further refines assessments using deterministic estimates of exposure and hazard, which are still conservative but incorporate more specific data. The assessment is further refined in Tier 2 with more realistic exposure scenarios and potentially more detailed hazard data, still using conservative assumptions but tailored to specific exposure scenarios. Finally, Tier 3 is the most detailed and data-intensive tier, using probabilistic

models and complex assessments such as physiologically based pharmacokinetic (PBPK) models to refine the risk characterization. Moreover, in some cases, additional uncertainty factors, due to certain specific vulnerabilities within a population that are not covered by standard uncertainty factors may be justified, e.g., developmental neurotoxic effects in children due to chlorpyrifos and permethrin.

At minimum, a global framework should implement screening-level cumulative assessment for transformation products sharing a common mechanism of action, by building on WHO/IPCS methodology. This would entail mechanism-based grouping, development of group reference values where warranted, and assessment of combined-exposure scenarios. Such integration would substantially improve protectiveness while remaining feasible across regulatory settings.

6. Vulnerable populations: children and infants

It is important to recognize that children and infants have potentially higher exposure to chemicals in drinking-water including to pesticides and their transformation products. The relative volume of water consumed increases as body weight decreases. Consequently, the potential exposure for a child and an infant is approximately 3 and 4.5 times greater, respectively, than that of an adult using WHO's default factors for body weight and drinking-water intake volumes for adults (60 kg, 2 litres/day), children (10 kg, 1 L/day) and infants (5 kg, 0.75 litres/day; see Section 8.2.2 of the GDWQ, WHO, 2022). For infants, the default relative allocation factor of 20 % may be increased to as much as 80 % if there is no significant additional exposure through the diet besides the preparation of milk bottles. Furthermore and as noted in Table 1 regarding Switzerland's adjustment of the TTC III value, infants are known to have 2 to 3 times lower renal metabolic function than adults (EFSA Scientific Committee et al., 2019). This is typically considered to be covered by the 10-fold uncertainty factor for toxicokinetic and toxicodynamic differences within species (EFSA Scientific Committee, 2012) and in the GDWQ, this is normally taken into account in long-term exposure in development of health-based guidance values. However, in some cases, e.g., if there are concerns regarding developmental neurotoxicity, an additional uncertainty factor may be justified when deriving health-based guidance values reflecting higher uncertainty around the specific endpoint or data quality. Hence, children and infants require explicit consideration in drinking water quality frameworks.

7. Alternative methods for risk assessment

In light of the limited availability of information and models tailored to nations outside the EU and North America, a novel approach to risk assessment involved the use of the PRIMET (Pesticide Risks in the Tropics for Man, Environment, and Trade) model, which leverages publicly accessible data on physico-chemical properties, toxicity, and pesticide use patterns for risk assessments (Teklu et al., 2023). The authors highlighted that, "*comparison of modelled PEC with actual measurement values of pesticides provides promising results for Ethiopia, although further large-scale studies are needed for full validation.*" It should also be noted that this approach focused on pesticides, not on transformation products. Presumably having fewer data for certain transformation products will increase the uncertainty of the predictions.

PRIMET was developed considering a specific scenario for the Ethiopian situation, namely the elevation, cropping pattern, rainfall and pesticide use status to select three representative locations that represent worst-case scenarios. The model calculates exposure concentration by using input parameters including pesticide application rate and frequency, physicochemical properties of the pesticide including DT50_Soil, DT50 water, molar mass, water solubility, vapour pressure and KOC. It also uses toxicological data including LC50 (mg/L), NOEC (mg/l) for fish; EC50 (mg/L)/ NOEC (mg/l) for *Daphnia*, EC50 (mg/L) for algae and macrophytes and (mg/kg_bw per day) for ARfD/ADI for human health.

Originally the tool was developed to support the pesticide registration system in Ethiopia to be a pioneer for Africa, following similar risk assessment procedures applied in the EU and US but adapting it to the specific Ethiopia situation by using specific Ethiopian meteorological, cropping pattern, soil and elevation data. Calculation of the exposure concentrations is performed with the Pesticides in Root Zone Model (PRZM) (for runoff) and Toxic Substances in Surface Waters (ToxSWa) (for fate in surface water) models, also used in the EU and USA for registration, using scenarios specifically tailored to Ethiopia (Adriaanse et al., 2015; Teklu et al., 2023; Wipfler et al., 2014).

O'Driscoll et al. (2022) developed an innovative risk ranking method to assess the health risks of pesticides in Irish drinking-water. This method integrates factors such as pesticide use, environmental persistence, mobility, and chronic health effects, providing a comprehensive tool for prioritizing pesticides in monitoring and risk assessments. Although the toxicological assessment is very simple, using a transformation product specific ADI if available, or the parent ADI if not, a key innovation is the inclusion of site-specific soil characteristics and the consideration of pesticide metabolites, which may be more toxic and persistent than their parent compounds. The study also performed sensitivity analyses, highlighting the significant impact of pesticide persistence and soil organic matter on risk scores, emphasizing the need for accurate data in assessments.

8. Conclusions and recommendations

This critical review reveals a significant and urgent gap in global regulatory coverage for pesticide transformation products in drinking water. The European Union is currently the only jurisdiction with formal frameworks for the assessment of pesticide transformation products in ground- and drinking-water. Guidance is less clear for so-called "non-relevant" transformation products, which has resulted in divergent assessments and limits in EU Member States. Many countries rely on limits as given in the GDWQ including for pesticides and some pesticide transformation products, but no international guidance has been developed for assessing transformation products in drinking-water. The absence of a global framework for evaluating pesticide transformation products in drinking-water and/or drinking-water sources has led many non-EU countries to adopt a more flexible approach. Some countries such as Australia, Brazil, Canada, Japan and New Zealand employ an *ad-hoc* assessment for specific pesticides and their transformation products and cite the JMPR assessment framework as standard, although this was actually developed for food residues.

An analysis of 12 guidance documents revealed a systematic, tiered approach across most schemes, ensuring a thorough and structured framework for the toxicological assessment of pesticide transformation products. Key strengths include the use of detailed, stepwise methodologies as seen in SANCO/221/2000, EFSA/ECHA, JMPR, JECFA, and SANCO/10,597/2003 assessment schemes, which provide clear processes for evaluating health risks. The tiered approaches used in SANCO/221/2000, Japan FSC, EFSA/ECHA guidance (EFSA, 2023) and SANCO/10,597/2003 start with *in silico* analyses and modelling followed by *in vitro* and *in vivo* tests. The level of investigation often depends on the concentration of the transformation products, but this approach may require significant expertise and resources, potentially limiting applicability in resource-constrained settings. For instance, frameworks that rely on higher tier exposure assessment (e.g., FOCUS type groundwater modelling or detailed drinking water exposure models) can be difficult to implement routinely because they require specialised modelling tools, and locally representative input data (e.g., climate/soil/hydrology, pesticide use patterns, and treatment-plant characteristics), which are not always available. Also, the US EPA, JMPR and JECFA emphasize evaluating the relative toxicity of metabolites compared to parent compounds, utilizing detailed toxicity screenings.

Handling data-poor substances presents a divergence among the documents. The US EPA frameworks, M7(R1), JMPR, JECFA, and EFSA/ECHA documents employ advanced predictive methods like (Q)SAR, read-across, and TTC to address data gaps, providing a scientific basis for decision-making when empirical data is limited or lacking. However, frameworks like SANCO/221/2000, Japan FSC and SANCO/10,597/2003 rely more heavily on *in vitro* and *in vivo* data. This reliance on empirical data highlights a potential weakness in managing risks for data-poor substances effectively.

Exposure considerations, including aggregate and cumulative exposure, and the use of monitoring data also vary significantly. The US EPA and EFSA/ECHA guidance documents provide comprehensive methodologies for quantifying exposure from multiple routes, emphasizing the importance of aggregate exposure. While considering aggregate exposure in its refined risk assessments, SANCO/221/2000 does not consider cumulative exposure, particularly regarding common transformation products. The use of monitoring data is increasingly emphasized for the refinement of risk assessments at higher tiers in the US EPA Framework for Conducting Pesticide Drinking-water Assessments for Surface Water. Conversely, the EFSA guidance, SANCO/221/2000, and Japan FSC mention monitoring data but lack detailed guidance. Additionally, only the EFSA/ECHA guidance (EFSA, 2023) provides a toxicological assessment scheme for water transformation products, while other documents do not address the impacts of water treatment processes, highlighting a gap in ensuring comprehensive risk assessments in treated drinking-water scenarios.

Recommendations

Based on the critical analysis presented above, we propose core elements for a pesticide TP assessment framework in drinking water that could complement and expand the WHO Guidelines for Drinking-Water Quality. The following recommendations can be made for the development of a toxicological assessment framework for pesticide transformation products in drinking-water that is flexible and scalable, allowing for adjustments based on available resources and expertise. This will ensure that the framework can be applied effectively in both resource-rich and resource-constrained settings.

1. Utilize a tiered, stepwise methodology that starts with *in silico* analyses and progresses to *in vitro* and *in vivo tests*. This approach should be scalable based on the (measured or predicted) concentration and frequency of occurrence of the transformation products, ensuring comprehensive assessment while optimizing resource use and minimizing animal use.
2. Implement advanced predictive methods such as (Q)SAR, read-across and grouping in conjunction with TTC to address data gaps. When possible, consideration could also be given to new approach methodologies. These methods provide a scientific basis for decision-making when empirical data is limited, ensuring a more robust assessment. To this end, data-sharing for the improvement of such models should also be promoted.
3. Ensure the scheme includes more detailed provisions for comparing the toxicity of transformation products with their parent compounds and/or other tested substances. This comparison should cover genotoxicity, carcinogenicity, reproductive and developmental toxicity, and systemic toxicity.
4. Provide clear guidance for differentiating between genotoxicity, carcinogenicity, developmental, reproductive and systemic toxicity. This ensures that all potential health risks are comprehensively evaluated.
5. Consider aggregate and cumulative exposure from multiple routes, including food and water.
6. Place data from exposure models and detected metabolite concentrations into context with respect to their representativeness for the catchment, plausibility in relation to land use, consistency with findings from other observation points, and the effectiveness of management options. This includes providing clear guidance for i) collecting, analysing, and integrating monitoring data to interpret exposure model results, and ii) designing monitoring programs based on expected metabolite occurrence and concentrations, considering pesticide use, substance properties, metabolite leaching behaviour, and local hydrological and agricultural conditions.
7. Consideration of how different water treatment methods affect the presence and toxicity of pesticide transformation products. Ideally this should cover common treatment processes like chlorination, ozonation, filtration, activated carbon adsorption and evaluate the formation of transformation products.

CRediT authorship contribution statement

Gyamfi Akyianu: Writing – review & editing, Writing – original draft, Methodology, Data curation. **Carsten Kneuer:** Writing – review & editing, Funding acquisition, Conceptualization. **Milou Dingemans:** Writing – review & editing, Methodology, Conceptualization. **Jennifer De France:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Bradley Lampe:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Helena Banning:** Writing – review & editing, Writing – original draft, Conceptualization. **John Fawell:** Writing – review & editing, Conceptualization. **Peter Jarvis:** Writing – review & editing, Writing – original draft, Conceptualization. **Berhan M. Teklu:** Writing – review & editing, Conceptualization. **Mari Asami:** Writing – review & editing, Data curation, Conceptualization. **Glenn Lurman:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Gyamfi Akyianu reports financial support was provided by World Health Organization. Reports a relationship with that includes:. Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envc.2026.101438.

Data availability

Data will be made available on request.

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